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(54) Human cardiac/brain tolloid-like protein

(57) HC/BTLP polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing hC/BTLP polypeptides and polynucleotides in the design of protocols for the treatment of restenosis, atherosclerosis, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), benign prostatic hypertrophy (BPH), nephritis, fibrosis, glomerulonephritis, gliosis, cirrhosis and anomalies of wound healing, such as keloids among others, and diagnostic assays for such conditions.

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Description

This application claims the benefit of U.S. Provisional Application No. 60/034,471, filed January 2, 1997.

5 FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to the astacin protein family, hereinafter referred to as human cardiac/brain tolloid-like protein (hC/BTLP). The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

15 The hC/BTLP gene appears to possess all of the important protein domains present in the bone morphogenetic protein (BMP)-1/procollagen C-proteinase (PCP) protein. Members of the astacin family of metalloproteinases, such as BMP-1, have previously been linked to cell differentiation and pattern formation during development through a proposed role in the activation of latent growth factors of the TGF- β superfamily. In addition, recent findings indicate that BMP-1 is identical to PCP, which is a metalloproteinase involved in the synthesis of matrix collagen. This observation suggests
20 that a functional link may exist between astacin metalloproteinases, growth factors and cell differentiation and pattern formation during development, as well as fibrotic processes characterized by the accumulation of matrix collagen.

Nucleotide and amino acid sequence homologues suggest that hC/BTLP, like BMP-1, possesses PCP activity. PCP activity is one of the essential enzymatic steps required for the extracellular production of insoluble collagen fibrils from soluble procollagen. However, mouse mammalian tolloid-like protein is the most closely related homologue of hC/BTLP. Mouse mammalian tolloid-like protein and BMP-1 are distinct gene products with differential tissue distribution. Based
25 on cross-species comparisons, the regulation and distribution of hC/BTLP would be expected to be distinct from BMP-1. Indeed, mouse mammalian tolloid-like protein exhibits a unique tissue distribution when compared to BMP-1. Thus, the selective inhibition of matrix collagen accumulation is important in highly localized fibrotic disorders, e.g., gliosis associated with neurotrauma and ventricular fibrosis associated with congestive heart failure. This indicates that the
30 astacin protein family has an established, proven history as therapeutic targets.

Clearly there is a need for identification and characterization of further members of the astacin protein family which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, restenosis, atherosclerosis congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), benign prostatic hypertrophy (BPH), nephritis, fibrosis, glomerulonephritis, gliosis, cirrhosis and anomalies of wound healing, such as
35 keloids, among others.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to hC/BTLP polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such hC/BTLP polypeptides and polynucleotides.
40 Such uses include the treatment of restenosis, atherosclerosis, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), benign prostatic hypertrophy (BPH), nephritis, fibrosis, glomerulonephritis, gliosis, cirrhosis and anomalies of wound healing, such as keloids, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with hC/BTLP imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic
45 assays for detecting diseases associated with inappropriate hC/BTLP activity or levels.

DESCRIPTION OF THE INVENTION**50 Definitions**

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"hC/BTLP" refers, among others, generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 or an allelic variant thereof.

"hC/BTLP activity or hC/BTLP polypeptide activity" or "biological activity of the hC/BTLP or hC/BTLP polypeptide" refers to the metabolic or physiologic function of said hC/BTLP including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of

said hC/BTLP.

"HC/BTLP gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library. "Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may

be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988)48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al.*, *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al.*, *J Molec Biol* (1990)215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

40 Polypeptides of the Invention

In one aspect, the present invention relates to hC/BTLP polypeptides (or hC/BTLP proteins). The hC/BTLP polypeptides include the polypeptide of SEQ ID NOS:2 and 4; as well as polypeptides comprising the amino acid sequence of SEQ ID NO: 2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within hC/BTLP polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and still more preferably at least 95% identity to SEQ ID NO:2. Furthermore, those with at least 97-99% are highly preferred. Preferably hC/BTLP polypeptide exhibit at least one biological activity of hC/BTLP.

The hC/BTLP polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

55 Fragments of the hC/BTLP polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned hC/BTLP polypeptides. As with hC/BTLP polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative exam-

ples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of hC/BTLP polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of hC/BTLP polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate hC/BTLP activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the hC/BTLP, including antigenic activity. Among the most preferred fragment is that having the amino acid sequence of SEQ ID NO: 4. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The hC/BTLP polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to hC/BTLP polynucleotides. hC/BTLP polynucleotides include isolated polynucleotides which encode the hC/BTLP polypeptides and fragments, and polynucleotides closely related thereto. More specifically, hC/BTLP polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding a hC/BTLP polypeptide of SEQ ID NO: 2, and polynucleotides having the particular sequences of SEQ ID NOS:1 and 3. hC/BTLP polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the hC/BTLP polypeptide of SEQ ID NO:2, and a polynucleotide comprising a nucleotide sequence that is at least 80% identical to that of SEQ ID NO:1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under hC/BTLP polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such hC/BTLP polynucleotides.

hC/BTLP of the invention is structurally related to other proteins of the astacin protein family, as shown by the results of sequencing the cDNA encoding hC/BTLP. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 252 to 3293) encoding a polypeptide of 1013 amino acids of SEQ ID NO:2. The amino acid sequence of Table 2 (SEQ ID NO:2) has about 93.4% identity (using BlastP) in 945 of 1012 amino acid residues with mus musculus (mouse) mammalian tolloid-like protein. GenBank Accession #U34042. The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 88.4% identity (using BlastN) in 2731 of 3089 nucleotide residues with mus musculus mammalian tolloid-like protein. GenBank Accession #U34042. Thus, hC/BTLP polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

Table 1^a

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1 CTTACCTGCC CTCCGCCCAC CCGTGGGCCC CTAGCCAAC TCTCCCTGCC

51 ACTGGGGGTA ACAGGCAGTG CTGCCCTCT CTACTGTCCC GGCGGCATCC

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101 ACATGTTTCC GGACACCTGA GCACCCCGGT CCGCCGAGG AgCCTCCGGG

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151 TGGGGAGAAg AgCACCGGTG CCCCTAGCCC CGCACATCAg CGGGACCGC
 201 GGCTGCCTAA CtTClGGGTG CGTCCCTC CTTTCCTCC GGGGAgGAg
 251 GATGGGGTTG GGAACgCTTT CCCCGAgGAT GCTCGTGTGG CTGGTGGCCT
 301 CGGGGATTGT TTTCTACGGG GA_gCTaTGGG TCTGCGCTGG CCTCgATTAT
 351 GATTACACTT TTGATGGGAA CgAAgAgGAT AAAACAGAGA CTATAGATTA
 401 CAAGGACCCG TGTAAGCCG CTGTATTTTG GGGCGATATT GCCTTAGATG
 451 ATGAAGACTT AAATATCTTT CAaATAGATA GGACAATTGA CCTTACGCAG
 501 AACCCCTTG GAAACCTTG ACATACCACA GGTGGACTTG GAGACCATGC
 551 TATGTCAAAG AAGCGAGGGG CCCTCTACCA ACTTATAGAC AGGATAAGAA
 601 GAATTGGCTT TGGCTTGGAG CAAAACAACA CAGTTAAGGG AAAAGTACCT
 651 CTACAATTCT CAGGGCAAAA TGAGAAAAAT cGAGTCCCA GAGCCGCTAC
 701 ATCAAGAACG GAAAGaTAT GGCCTGGAGG CGTTATTCCT TATGTTATAG
 751 GAGGaAACTT CACTGCCAGC CAGAGAGCCA TGTTCaAGCA GGCCATGAGG
 801 CACTGGGaAA AGCACACATG TGTGACTTTC ATAGAAAGAA GTGATGAAGA
 851 GAGTTACATT GTATTCACCT ATAGGCCTTG TGGATGCTGC TCCTATGTAG
 901 GTcGGCGAGG AAgTGGACCT CAGGCAATCT CTATCGGCAA GAACTGTGAT
 951 AAATTGGGA TtGTtGTTCA TGAATTGGGT CatGTGATAG GCTTTTGGCA
 1001 TGAACACACA AGACCAGATC GAGATAACCA CGTAACTATC ATAaGAGAAA
 1051 ACATCCAGCC AGGTCAAgAG TACAATTTTC TGAaGATGGA GCCTGGAGAA
 1101 GcAAACTCAC TTGGAGAAAG ATATGATTTC GACAGTATCA TGCATATGC
 1151 CAGGAACaCC TTCTCAAgGG GGATGTTTct GGATACCATT CTCCCCTCCC
 1201 GTGATGATAA TGGCaTACGT CctGCAATTG GTCAGCgAAC CCGTCTAAGC
 1251 aAAGGAgATA TCgCaCAGGC AAGAAAGCTG TATAGATGTC CAGCATGTGG
 1301 AGAAACTcTA CAAGAATCCA ATGGCAACCT TTCCTCTCCA GGATTTCaCC
 1351 ATGGCTACCC TTCTTACACA CACTGCATCT GGAGAGTTTC TGTGACCCCA
 1401 GGGGAGAAGA TTGTTTTAAA TTTTACAACG ATGGATCTAT ACAAGAGTAG
 1451 TTTGTGCTGG TATGACTATA TTGAAGTAAG AGACGGGTAC TGGAGAAAAT
 1501 CACCTCTCCT TGgTAGATTC TGTGGGGACA AATtGCCTGA AGTTCTTACT

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1551 TCTACAGACA GCAGAATGTG GATTGAGTTT CGTAGCAGCA GTAATTGGGT
1601 AGGAAAAGGC TTTGCAGCTG TCTATGAAGC GATCTGTGGA GGTGAGATAC
1651 GTAAAAATGA AGGACAGATT CAGTCTCCCA ATTATCCTGA TGAATATCGC
1701 CCGATGAAGG AATGTGTGTG GAAAATAACA GTGTCTGAGA GCTACCACGT
1751 CGGGCTGACC TTTCACTCCT TTGAGATTGA AAGACATGAC AATTGTGCTT
1801 ATGACTACCT GGAAGTTAGA GATGGAACCA GTGAAAATAG CCCTTTGATA
1851 GGGCGTTTCT GTGGTTATGA CAAACCTGAA GACATAAGAT CTACCTCCAA
1901 TACTTTGTGG ATGAAGTTTG TTTCTGACGG AACTGTGAAC AAAGCAGGGT
1951 TTGCTGCTAA CTTTTTTAAA GAGGAAGATG AGTGTGCCAA ACCTGACCGT
2001 GGAGGCTGTG AGCAGCGATG TCTGAACACT CTGGGCAGTT ACCAGTGTGC
2051 CTGTGAGCCT GGCTATGAGC TGGGCCCAAG CAGAAGGAGC TGTGAAGCTG
2101 CTTGTGGTGG ACTTCTTACC AAACCTAAGC GCACCATAAC CACCCCTGGC
2151 TGGCCCAAGG AGTACCCTCC TAATAAGAAC TGTGTGTGGC AAGTGGTTGC
2201 ACCAACCAG TACAGAATTT CTGTGAAGTT TGAGTTTTTT GAATTGAAG
2251 GCAATGAAGT TTGCAAATAT GATTATGTGG AGATCTGGAG TGGTCTTTCC
2301 TCTGAGTCTA AACTGCATGG CAAATTCTGT GCGCTGAAG TGCCTGAAGT
2351 GATCATATCC CAGTTCAACA ATATGAGAAT TGAATTCAAA TCTGACAATA
2401 CTGTATCCAA GAAGGGCTTC AAAGCACATT TTTTCTCAGA CAAAGATGAA
2451 TGCTCTAAGG ATAATGGTGG ATGTCAGCAC GAATGTGTCA ACACCATGGG
2501 GAGCTACATG TGTCAATGCC GTAATGGATT TGTGCTACAT GACAATAAAC
2551 ATGATTGCAA GGAAGCTGAG TGTGAACAGA AGATCCACAG TCCAAGTGGC
2601 CTCATCACCA GTCCCAACTG GCCAGACAAG TACCCAAGCA GGAAAGAATG
2651 CACTTGGGAA ATCAGCGCCA CTCCTGGCCA CCGAATCAAA TTAGCCTTTA
2701 GTGAATTGGA GATTGAGCAG CATCAAGAAT GTGCTTATGA CCACTTAGAA
2751 GTATTTGATG GAGAAACAGA AAAGTCACCG ATTCTTGGAC GACTATGTGG
2801 CAACAAGATA CCAGATCCCC TTGTGGCTAC TGGAAATAAA ATGTTTGTTT
2851 GGTTTGTTC TGAIGCATCT GTTCAAAGAA AAGGCTTTCA AGCCACACAT
2901 TCTACAGAGT GTGGCGGACG ATTGAAAGCA GAATCAAAAC CAAGAGATCT

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2951 GTACTCACAT GCTCAGTTTG GTGATAACAA CTACCCAGGA CAGGTTGACT
3001 GTGAATGGCT ATTAGTATCA GAACGGGGCT CTCGACTTGA ATTATCCTTC
3051 CAGACATTG AAGTGGAGGA AGAAGCGGAC TGTGGCTATG ACTATGTGGA
3101 GCTCTTTGAT GGTCTTGATT CAACAGCTGT GGGGCTTGGT CGATTCTGTG
3151 GATCCGGGCC ACCAGAAGAG ATTTATTCAA TTGGAGATTC AGTTTAAATT
3201 CATTTCACACA CTGATGACAC AATCAACAAG AAGGGATTTC ATATAAGATA
3251 CAAAAGCATA AGATATCCAG ATACCACACA TACCAAAAAA TAACACCAAA
3301 ACCTCTGTCA GAACACAAAG GAATGTGCAT AATGGAGAGA AGACATATTT
3351 TTTTAAAAC TGAAGATATT GGCACAAATG TTTTATACAA AGAGTTTGAA
3401 CAAAAATCC CTGTAAGACC AGAATTATCT TTGTACTAAA AGAGAAGTTT
3451 CCAGCAAAAC CCTCATCAGC ATTACAAGGA TATTTGAACT CCATGCTTGA
3501 TGGTATTAAT AAAGCTGGTG AAAGGGCATC ATATACTTCA AGGAAGACTC
3551 TACAAGCTTT TGTTCACAGC TTGAAATAGA TGCCTCACAA TTCAGACAGT
3601 TTAATTCAGG AACTGTGACC CTGAAGTGTT CTTTTTGACA ATTTGTCAAG
3651 ATTTAGGGAC ATAAATGAT CTTCAGGTC GTAACTGGA AAACAGTATT
3701 TTGGTTGTCT TAGGATAATT GCTGACTTTG TATCTTGGAT ACAGTGATAA
3751 CCAGATCCAT ATAAGGTGAA TGTGAAATGG GAGTCTTCTG AGGGTGATTT
3801 GTACTTTCCA TGTGTATGTG TGTGTCTGGT GTTTGGAAAC TGGGATATTT
3851 CAGCTTCATT ATTTCCACTT GCAGGCCAGC TTAACCTCTG AAACACAAAT
3901 GATCTTGAGA CCACTTAGT GTACTTACAT TTAGATGAGT TTGAAATCTC
3951 AATGGTGTCT AATTATTGCA GTTAAATCT AGACATCAGT TCTTTAAGTC
4001 TCAGAAAAAG CCCAGTGAAT TGGTAACTT AGTTCTTTTT TTTGGAAGTG
4051 CTGCCTTTTC ACACCAAATC CAAGAAGCCT GTGATGTCTT ATGAACCTTA
4101 TGAGAAAACT CGAAGAGGT GTGAGCAGGA TTCTTCTGAA TGACTGTCTG
4151 GATGGTTCAT TACTCAAGTT ACTGCTGCTG CTATTGTCTT TCCTTTGTTG
4201 TCGATCTGTT ATTGTTGTAT TATTATTGTT GATGTTGTCA TGGTTAATCT
4251 ATTTTTTAAA ATTGAAATGA AGCAGAAGTA GGCCTTGTGA GAACTGAAAG
4301 GTCTCTTCA TTTTCTCTT CCTGGGATTC ATTTTTTCAA AACACAATGC

4351 TGGAAAAAA AGATTGTTT CTGAAAGACT TCTATGGTG CTATTCCATA
 4401 AACTTTTTT CAAACAAGT TTTGACCTT GAGCCAACCC ACCCGTAGAC
 4451 TACGAATGTC TCCCTATGGC TGGTAGCATT TGAAGACTAA AGACTTGTC
 4501 AATATATCAA GAGTATATCA TTGCAAGGGC AGCACTTGT C CTGTGGAACA
 4551 ACTACTTATA ATGCCTTAGA ATTCTGCAC ATGATCAAAC AGATCCTCCT
 4601 AAAACACACC TTTTGAATG TTGAACATAA TAGTGTATGT TAATTAACAG
 4651 CTCTATGAAG AAAATCCATT TCCATGACTG AAGCATTGGA TATAAATATG
 4701 GTGTCTGCT TTTTTGTAG AAAATGTAAT TTGAGGATGA ATTTTCTGCT
 4751 TTAAAGGCAT GTGTGTTTT AAAATTAATG AATGTAGATG TGTGATTGTC
 4801 TGAGTGAGTG AAATACAAG AGGTAAAAA TAATGGGTGG TTGAAAAGTT
 4851 AAAATGTATG TGCCAAGTTC TACTAGAATT CCATTGAAA TAGCACCTTC
 4901 CTTAGGTTTC ATGGACAAAT AATGGGAAT TCTAATTTTG ATCAATCCCA
 4951 TTAAAAAAG GCTCTTCCT TTAGAGAAAC TCTATTTGA TGTCAATATA
 5001 GATTACTGTA TGAAGTAGCT TTGTGTCTGT TACCTGTCCA TGAGCATACA
 5051 ACATTGAATA CAATGGGTG TATTCCTTCA GTTTACACA ATTAAAGTAT
 5101 ACACACAGAT GTAAAAAAA AAAAAAAAAA AAAAAAAC TCGAG

^a A nucleotide sequence of a hC/BTLP (SEQ ID NO: 1).

Table 2^b

1 MGLGTLSPRM LVWLVASGIV FYGELWVCAG LDYDYTFDGN EEDKTETIDY
 51 KDPCKAAVFW GDIALDDEDL NIFQIDRTID LTQNPFGNLG HTTGGLGDHA
 101 MSKRGALYQ LIDRIRRIGF GLEQNNTVKG KVPLQFSGQN EKNRVPRAAT
 151 SRTERVWPGG VIPYVIGGNF TGSQRAMFKQ AMRHWEKHTC VTFIERSDEE
 201 SYIVFTYRPC GCCSYVGRRG SGPQAISIGK NCDKFGIVVH ELGHVIGFWH
 251 EHTRPDRDNH VTIIRENIQP GQEYNFLKME PGEANSLGER YDFDSIMHYA
 301 RNTFSRGMFL DTILPSRDDN GIRPAIGQRT RLSKGDIAQA RKLYRCPACG
 351 ETLQESNGNL SSPGFPNGYP SYTHCIWRVS VTPGEKIVLN FTIMDLKSS

5 401 LCWYDYIEVR DGYWRKSPLL GRFCGDKLPE VLTSTDSRMW IEFSSSNWV
 451 GKGFAAVYEA ICGGEIRKNE GQIQSPNYPD DYRPMKECVW KITVSESYHV
 501 GLTFQSFEIE RHDNCAYDYL EVRDGTSENS PLIGRFCGYD KPEDIRSTSN
 10 551 TLWMKFVSDG TVNKAGFAAN FFKEEDECAK PDRGGCEQRC LNTLGSYQCA
 601 CEPGYELGPD RRSCEAACGG LLTKLNGTIT TPGWPKEYPP NKNCVWQVVA
 15 651 PTQYRISVKF EFFELEGNEV CKYDYVEIWS GLSSESCLHG KFCGAEVPEV
 701 ITSQFNMRI EFKSDNTVSK KGFKAHFFSD KDECCKDNGG CQHECVNTMG
 751 SYMCQCRNGF VLHDNKHDKK EAECEQKIHS PSLITSPNW PDKYPSRKEC
 20 801 TWEISATPGH RIKLAFSEFE IEQHQECAYD HLEVFDGETE KSPILGRLCG
 851 NKIPDPLVAT GNKMFVRFVS DASVQRKGFQ ATHSTECGGR LKAESKPRDL
 25 901 YSHAQFGDNN YPGQVDCWL LVSEGRSRLE LSFQTFEVEE EADCGYDYVE
 951 LFDGLDSTAV GLGRFCGSGP PEEIYSIGDS VLIHFHTDDT INKKGPHIRY
 1001 KSIRYPDTTH TTK

30 An amino acid sequence of a hC/BTLP (SEQ ID NO: 2).

35 One polynucleotide of the present invention encoding hC/BTLP may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human 8 week old human embryo using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature*, (1992) 355:632-634; Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

40 The nucleotide sequence encoding hC/BTLP polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 252 to 3293 of SEQ ID NO:1), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

45 When the polynucleotides of the invention are used for the recombinant production of hC/BTLP polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself, the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexahistidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al., Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The

50 polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.
 Further preferred embodiments are polynucleotides encoding hC/BTLP variants comprising the amino acid sequence of hC/BTLP polypeptide of Table 2 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination. Among the preferred polynucleotides of the present invention is contained in Table 3 (SEQ ID NO: 3) encoding the amino acid sequence of Table 4 (SEQ ID NO: 4).

Table 3^c

5	GAATTGGCA CGAGCTCGTG CGCTCGTGC CGGGGTACT GGAGAAATC ACCTCTCCTT	60
	GATTCTGTGG GGACAAATTG CCTGAAGTTC TTACTTCTAC AGACAGCAGA ATGTGGATTG	120
10	AGTTTCGTAG CAGCAGTAAT TGGGTAGGAA AAGGCTTTGC AGCTGTCTAT GAAGCGATCT	180
	GTGGAGGTGA GATACGTAAA AATGAAGGAC AGATTCACTC TCCCAATTAT CCTGATGACT	240
	ATGCCCCGAT GAAAGAATGT GTGTGGAAAA TAACAGTGTCT TGAGAGCTAC CACGTGGGC	300
	TGACCTTTCA GTCCTTTGAG ATTGAAAGAC ATGACAATTG TGCTTATGAC TACCTGGAAG	360
15	TTAGAGATGG AACCACTGAA AATAGCCCTT TGATAGGGCG TTTCTGTGGT TATGACAAAC	420
	CTGAAGACAT AAGATCTACC TCCAATACTT TGTGGATGAA GTTTGTTTCT GACGGAACCTG	480
	TGAACAAAGC AGGGTTTGCT GCTAACTTTT TTAAGAGGA AGATGAGTGT GCCAAACCTG	540
	ACCGTGGAGG CTGTGAGCAG CGATGTCTGA AACTCTGGG CAGTTACCAG TGTGCCTGTG	600
20	AGCCTGGCTA TGAGCTGGGC CCAGACAGAA GGAGCTGTGA AGCTGCTTGT GGTGGACTTC	660
	TTACCAAACCT TAACGGCACC ATAACCACC CTGGCTGGCC CAAGGAGTAC CCTCCTAATA	720
	AGAACTGTGT GTGGCAAGTG GTTGACCAA CCCAGTACAG AATTTCTGTG AAGTTTGAGT	780
	TTTTTGAATT GGAAGGCAAT GAAGTTGCA AATATGATTA TGTGGAGATC TGGAGTGGTC	840
25	TTTCTCTGA GTCTAAACTG CATGGCAAAT TCTGTGGGCG TGAAGTGCCT GAAGTGATCA	900
	CATCCCAGTT CAACAATATG AGAATTGAAT TCAAATCTGA CAATACTGTA TCCAAGAAGG	960
	GCTTCAAAGC ACATTTTTTC TCAGACAAAG ATGAATGCTC TAAGGATAAT GGTGGATGTC	1020
	AGCAGCAATG TGTCAACAG ATGGGGAGCT ACATGTGTCA ATGCGTAAT GGATTTGTGC	1080
30	TACATGACAA TAAACATGAT TGCAAGGAAG CTGAGTGTGA ACAGAAGATC CACAGTCCAA	1140
	GTGGCCTCAT CACCACTCCC AACTGGCCAG ACAAGTACCC AAGCAGGAAA GAATGCACTT	1200
	GGGAAATCAG CGCACTCCT GGCCACCGAA TCAAATTAGC CTTTAGTGAA TTTGAGATTG	1260
	AGCAGCATCG GGAATGTGCT TATGACCACT TAGAAGTATT TGATGGAGAA ACAGAAAAGT	1320
35	CACCGATTCT TGGACGACTA TGTGGCAACA AGATACCAGA TCCCCTTGTG GCTACTGGAA	1380
	ATAAAATGTT TGTTGGTTT GTTTCTGATG CATCTGTTCA AAGAAAAGGC TTTCAAGCCA	1440
	CACATTCTAC AGAGTGTGGC GGACGATTGA AAGCAGAATC AAAACCAAGA GATCTGTACT	1500

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5	CACATGCTCA GTTTGGTGAT AACAACTACC CAGGACAGGT TGACTGTGAA TGGCTATTAG	1560
	TATCAGAAAG GGGCTCTCGA CTGAATTAT CCTTCCAGAC ATTTGAAGTG GAGGAAGAAG	1620
	CAGACTGTGG CTATGACTAT GTGGAGCTCT TTGATGGTCT TGATTCAACA GCTGTGGGGC	1680
	TTGGTGGATT CTGTGGATCC GGGCCACCAG AAGAGATTTA TTCAATTGGA GATTCAGTTT	1740
	TAATTCAATT CCACACTGAT GACACAATCA ACAAGAAGGG ATTTCATATA AGATACAAAA	1800
10	GCATAAGATA TCCAGATACC ACACATACCA AAAAATAACA CCAAAACCTC TGTGAGAACA	1860
	CAAAGGAATG TGCATAATGG AGAGAAGACA TATTTTTTTT AAAACTGAAG ATATTGGCAC	1920
	AAATGTTTTA TACAAAGAGT TTGAACAAAA AATCCCTGTA AGACCAGAAT TATCTTTGTA	1980
	CTAAAAGAGA AGTTTCCAGC AAAACCCTCA TCAGCATTAC AAGGATATTT GAACTCCATG	2040
15	CTTGATGGTA TTAATAAAGC TGGTGAAAGG GCATCATATA CTTCAAGGAA GACTCTACAA	2100
	GCTTTTGTTT ACAGCTTGAA ATAGATGCCT CACAATTGAG ACAGTTTAAAT TCAGGAACTG	2160
	TGACCCTGAA GTGTTCTTTT TGACAATTG TCAAGATTTA GGGACATAAA ATGATCTTGC	2220
	AGGTCTGAAA CTGGAACACA GTATTTTGGT TGTCTAGGA TAATTGCTGA CTTTGTATCT	2280
20	TGGATACAGT GTAAACCAGA TCCATATAAG GTGAATGTGA AATGGGAGTCT TCTGAGGGT	2340
	GATTGTACT TTCCATGTGT ATGTGTGTGT CTGGTGTG GAAACTGGGA TATTTGAGT	2400
	TCATTATTTT CACTTGCAGG CCAGCTTAAC CTCTGAAACA CAAATGATCT TGAGACCACT	2460
	TTAGTGACT TACATTTAGA TGAGTTTGAA ATCTCAATGG TGTCTAATTA TTGCAGTTAA	2520
25	ATTCTAGACA TCAGTTCTTT AAGTCTCAGA AAAAGCCAG TGAATTGGTA AACTTAGTTT	2580
	TTTTTTTTGG AAGTGCTGCC TTTTCACACC AAATCCAAGA AGCCTGTGAT GTCTTATGAA	2640
	CCTTATGAGA AAACCTCGAA GAGGTGTGAG CAGGATTTCT CTGAATGACT GTCTGGATGG	2700
	TTCAATCTC AAGTACTGCT TGCTGCTATT GTCTTCTT TGTGTGAT CTGTTATTGT	2760
	TGTATTATTA TTGTTGATGT TGTCTGGT AATCTATTTT TTTAAATTGA AATGAAGCAG	2820
30	AAGTAGGCTT TGTGAGAACT GAAAGGTCT TTTCAATTTT CTCTCTGG GATTCAATTT	2880
	TTCAAAACAC AATGCTGGAA AAAAAGATT TGTTCTGAA AGACTCTTA TGGTGCTATT	2940
	CCATAAACTT TTTTCAAAC AAGTTTTTGA CCTTTGAGCC AACCACCCG TAGACTACGA	3000
	ATGTCTCCCT ATGGCTGGTA GCATTTGAAG ACTAAGACT TGTCAAATAT ATCAAGAGTA	3060
35	TATCATTGCA AGGGCAGCAC TTGTCTGTG GAACAACCTAC TTATAATGCC TTAGAATTCC	3120
	TGCACATGAT CAAACAGATC CTCTAAAAC ACACCTTTG AAATGTTGAA CATAATAGTG	3180
	TATGTTAATT AACAGCTCTA TGAAGAAAAT CCATTTCCAT GACTGAAGCA TTGGATATAA	3240
	ATATGGTGTCT CTGCTTTTTT TGTAGAAAAT GTAATTTGAG GATGAATTTT CTGCTTTAAA	3300
	GGCATGTGTG TTTTAAAAAT TAATGAATGT AGATGTGTGA TTGTCTGAGT GAGTGAACT	3360
40	ACAAGAGGTA AAAAATAATG GGTGGTTGAA AAGTTAAAAAT GTATGTGCCA AGTTCTACTA	3420
	GAATTCATT TGAAATAGCA CCTTCTTAG GTTCATGGA CAAATAATGG GAACTCTAA	3480
	TTTTGATCAA TCCCATTAATA AAAAGGCTCT TTCCTTTAGA GAACTCTAT TTGATGTCA	3540
	ATATAGATTA CTGTATGAAG TAGCTTTGTG TCTGTTACCT GTCCATGAGC ATACAACATT	3600
45	GAATACAATT GGGTGTATTCT TTTCAATTTT ACACAATTAA AGTATACACA CAGATGTAAA	3660
	AAAAAAAAAA AAAAAAAAAA AAAACTCGAG	3690

A partial nucleotide sequence of a hC/BTLP (SEQ ID NO: 3).

Table 4^d

5 Phe Cys Gly Asp Lys Leu Pro Glu Val Leu Thr Ser Thr Asp Ser Arg
 1 5 10 15
 Met Trp Ile Glu Phe Arg Ser Ser Ser Asn Trp Val Gly Lys Gly Phe
 20 25 30
 10 Ala Ala Val Tyr Glu Ala Ile Cys Gly Gly Glu Ile Arg Lys Asn Glu
 35 40 45
 Gly Gln Ile Gln Ser Pro Asn Tyr Pro Asp Asp Tyr Arg Pro Met Lys
 50 55 60
 15 Glu Cys Val Trp Lys Ile Thr Val Ser Glu Ser Tyr His Val Gly Leu
 65 70 75 80
 Thr Phe Gln Ser Phe Glu Ile Glu Arg His Asp Asn Cys Ala Tyr Asp
 85 90 95
 Tyr Leu Glu Val Arg Asp Gly Thr Ser Glu Asn Ser Pro Leu Ile Gly
 100 105 110
 20 Arg Phe Cys Gly Tyr Asp Lys Pro Glu Asp Ile Arg Ser Thr Ser Asn
 115 120 125
 Thr Leu Trp Met Lys Phe Val Ser Asp Gly Thr Val Asn Lys Ala Gly
 130 135 140
 25 Phe Ala Ala Asn Phe Phe Lys Glu Glu Asp Glu Cys Ala Lys Pro Asp
 145 150 155 160
 Arg Gly Gly Cys Glu Gln Arg Cys Leu Asn Thr Leu Gly Ser Tyr Gln
 165 170 175
 30 Cys Ala Cys Glu Pro Gly Tyr Glu Leu Gly Pro Asp Arg Arg Ser Cys
 180 185 190
 Glu Ala Ala Cys Gly Gly Leu Leu Thr Lys Leu Asn Gly Thr Ile Thr
 195 200 205
 35 Thr Pro Gly Trp Pro Lys Glu Tyr Pro Pro Asn Lys Asn Cys Val Trp
 210 215 220
 Gln Val Val Ala Pro Thr Gln Tyr Arg Ile Ser Val Lys Phe Glu Phe
 225 230 235 240
 40 Phe Glu Leu Glu Gly Asn Glu Val Cys Lys Tyr Asp Tyr Val Glu Ile
 245 250 255
 Trp Ser Gly Leu Ser Ser Glu Ser Lys Leu His Gly Lys Phe Cys Gly
 260 265 270
 45 Ala Glu Val Pro Glu Val Ile Thr Ser Gln Phe Asn Asn Met Arg Ile
 275 280 285
 Glu Phe Lys Ser Asp Asn Thr Val Ser Lys Lys Gly Phe Lys Ala His
 290 295 300
 50 Phe Phe Ser Asp Lys Asp Glu Cys Ser Lys Asp Asn Gly Gly Cys Gln
 305 310 315 320
 His Glu Cys Val Asn Thr Met Gly Ser Tyr Met Cys Gln Cys Arg Asn
 325 330 335

Gly Phe Val Leu His Asp Asn Lys His Asp Cys Lys Glu Ala Glu Cys
 340 345 350
 Glu Gln Lys Ile His Ser Pro Ser Gly Leu Ile Thr Ser Pro Asn Trp
 355 360 365
 Pro Asp Lys Tyr Pro Ser Arg Lys Glu Cys Thr Trp Glu Ile Ser Ala
 370 375 380
 Thr Pro Gly His Arg Ile Lys Leu Ala Phe Ser Glu Phe Glu Ile Glu
 385 390 395 400
 Gln His Arg Glu Cys Ala Tyr Asp His Leu Glu Val Phe Asp Gly Glu
 405 410 415
 Thr Glu Lys Ser Pro Ile Leu Gly Arg Leu Cys Gly Asn Lys Ile Pro
 420 425 430
 Asp Pro Leu Val Ala Thr Gly Asn Lys Met Phe Val Arg Phe Val Ser
 435 440 445
 Asp Ala Ser Val Gln Arg Lys Gly Phe Gln Ala Thr His Ser Thr Glu
 450 455 460
 Cys Gly Gly Arg Leu Lys Ala Glu Ser Lys Pro Arg Asp Leu Tyr Ser
 465 470 475 480
 His Ala Gln Phe Gly Asp Asn Asn Tyr Pro Gly Gln Val Asp Cys Glu
 485 490 495
 Trp Leu Leu Val Ser Glu Arg Gly Ser Arg Leu Glu Leu Ser Phe Gln
 500 505 510
 Thr Phe Glu Val Glu Glu Glu Ala Asp Cys Gly Tyr Asp Tyr Val Glu
 515 520 525
 Leu Phe Asp Gly Leu Asp Ser Thr Ala Val Gly Leu Gly Arg Phe Cys
 530 535 540
 Gly Ser Gly Pro Pro Glu Glu Ile Tyr Ser Ile Gly Asp Ser Val Leu
 545 550 555 560
 Ile His Phe His Thr Asp Asp Thr Ile Asn Lys Lys Gly Phe His Ile
 565 570 575
 Arg Tyr Lys Ser Ile Arg Tyr Pro Asp Thr Thr His Thr Lys Lys
 580 585 590

A partial amino acid sequence of a hC/BTLP (SEQ ID NO: 4).

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably 97-99% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof (including that of SEQ ID NO:3), may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding hC/BTLP polypeptide and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human) that have a high sequence similarity to the hC/BTLP gene. Such hybridization techniques are known to those of skill in

the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

5 In one embodiment, to obtain a polynucleotide encoding hC/BTLP polypeptide, including homologs and orthologs from species other than human, comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof (including that of SEQ ID NO: 3), and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Thus in another aspect, hC/BTLP polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof (including that of SEQ ID NO: 3). Also included with hC/BTLP polypeptides are polypeptide comprising amino acid sequence encoded by nucleotide sequence obtained by the above hybridization condition. Stringent hybridization conditions are as defined above or, alternatively, conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

20 Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

50 If the hC/BTLP polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If hC/BTLP polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered. hC/BTLP polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is

denatured during isolation and or purification.

Diagnostic Assays

5 This invention also relates to the use of hC/BTLP polynucleotides for use as diagnostic reagents. Detection of a mutated form of hC/BTLP gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of hC/BTLP. Individuals carrying mutations in the hC/BTLP gene may be detected at the DNA level by a variety of techniques.

10 Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled hC/BTLP nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising hC/BTLP nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee *et al.*, *Science*, Vol 274, pp 610-613 (1996)).

25 The diagnostic assays offer a process for diagnosing or determining a susceptibility to restenosis, atherosclerosis, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), benign prostatic hypertrophy (BPH), nephritis, fibrosis, glomerulonephritis, gliosis, cirrhosis and anomalies of wound healing, such as keloids through detection of mutation in the hC/BTLP gene by the methods described.

30 In addition, restenosis, atherosclerosis, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), benign prostatic hypertrophy (BPH), nephritis, fibrosis, glomerulonephritis, gliosis, cirrhosis and anomalies of wound healing, such as keloids, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of hC/BTLP polypeptide or hC/BTLP mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an hC/BTLP polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

35 Thus in another aspect, the present invention relates to a diagnostic kit for a disease or susceptibility to a disease, particularly restenosis, atherosclerosis, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), benign prostatic hypertrophy (BPH), nephritis, fibrosis, glomerulonephritis, gliosis, cirrhosis and anomalies of wound healing, such as keloids, which comprises:

- (a) a hC/BTLP polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof;
 - (b) a nucleotide sequence complementary to that of (a);
 - 45 (c) a hC/BTLP polypeptide, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or
 - (d) an antibody to a hC/BTLP polypeptide, preferably to the polypeptide of SEQ ID NO: 2.
- It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Chromosome Assays

50 The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). The differences

in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

5 Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the hC/BTLP polypeptides. The term "immunospecific" means that the antibodies have substantial greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the hC/BTLP polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against hC/BTLP polypeptides may also be employed to treat restenosis, atherosclerosis, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), benign prostatic hypertrophy (BPH), nephritis, fibrosis, glomerulonephritis, gliosis, cirrhosis and anomalies of wound healing, such as keloids, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with hC/BTLP polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from restenosis, atherosclerosis, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), benign prostatic hypertrophy (BPH), nephritis, fibrosis, glomerulonephritis, gliosis, cirrhosis and anomalies of wound healing, such as keloids, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering hC/BTLP polypeptide via a vector directing expression of hC/BTLP polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a hC/BTLP polypeptide wherein the composition comprises a hC/BTLP polypeptide or hC/BTLP gene. The vaccine formulation may further comprise a suitable carrier. Since hC/BTLP polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

The hC/BTLP polypeptide of the present invention may be employed in a screening process for compounds which activate (agonists) or inhibit activation of (antagonists, or otherwise called inhibitors) the hC/BTLP polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess identify agonist or antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These agonists or antagonists may be natural or modified substrates, ligands, enzymes, receptors, etc., as the case may be, of the polypeptide of the present invention; or may be structural or functional mimetics of the polypeptide of the present invention. See Col-

igan et al., *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

HC/BTLP polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate hC/BTLP polypeptide on the one hand and which can inhibit the function of hC/BTLP polypeptide on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as restenosis, atherosclerosis, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), benign prostatic hypertrophy (BPH), nephritis, fibrosis, glomerulonephritis, gliosis, cirrhosis and anomalies of wound healing, such as keloids. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as restenosis, atherosclerosis, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), benign prostatic hypertrophy (BPH), nephritis, fibrosis, glomerulonephritis, gliosis, cirrhosis and anomalies of wound healing, such as keloids.

In general, such screening procedures may involve using appropriate cells which express the hC/BTLP polypeptide or respond to hC/BTLP polypeptide of the present invention. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells which express the hC/BTLP polypeptide (or cell membrane containing the expressed polypeptide) or respond to hC/BTLP polypeptide are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The ability of the cells which were contacted with the candidate compounds is compared with the same cells which were not contacted for hC/BTLP activity.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the hC/BTLP polypeptide is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the hC/BTLP polypeptide, using detection systems appropriate to the cells bearing the hC/BTLP polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed.

Further, the assays may simply comprise the steps of mixing a candidate compound with a solution containing a hC/BTLP polypeptide to form a mixture, measuring hC/BTLP activity in the mixture, and comparing the hC/BTLP activity of the mixture to a standard.

The hC/BTLP cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of hC/BTLP mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of hC/BTLP protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents which may inhibit or enhance the production of hC/BTLP (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The hC/BTLP protein may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. These include, but are not limited to, ligand binding and crosslinking assays in which the hC/BTLP is labeled with a radioactive isotope (eg 125I), chemically modified (eg biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (cells, cell membranes, cell supernatants, tissue extracts, bodily fluids). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. In addition to being used for purification and cloning of the receptor, these binding assays can be used to identify agonists and antagonists of hC/BTLP which compete with the binding of hC/BTLP to its receptors, if any. Standard methods for conducting screening assays are well understood in the art.

Examples of potential hC/BTLP polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, enzymes, receptors, etc., as the case may be, of the hC/BTLP polypeptide, e.g., a fragment of the ligands, substrates, enzymes, receptors, etc.; or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for hC/BTLP polypeptides; or compounds which decrease or enhance the production of hC/BTLP polypeptides, which comprises:

- (a) a hC/BTLP polypeptide, preferably that of SEQ ID NO:2;
 - (b) a recombinant cell expressing a hC/BTLP polypeptide, preferably that of SEQ ID NO:2;
 - (c) a cell membrane expressing a hC/BTLP polypeptide; preferably that of SEQ ID NO: 2; or
 - (d) antibody to a hC/BTLP polypeptide, preferably that of SEQ ID NO: 2.
- It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

55 Prophylactic and Therapeutic Methods

This invention provides methods of treating abnormal conditions such as, restenosis, atherosclerosis, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), benign prostatic hypertrophy (BPH), nephritis,

fibrosis, glomerulonephritis, gliosis, cirrhosis and anomalies of wound healing, such as keloids, related to both an excess of and insufficient amounts of hC/BTLP polypeptide activity.

If the activity of hC/BTLP polypeptide is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit the function of the hC/BTLP polypeptide, such as, for example, by blocking the binding of ligands, substrates, enzymes, receptors, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of hC/BTLP polypeptides still capable of binding the ligand, substrate, enzymes, receptors, etc. in competition with endogenous hC/BTLP polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the hC/BTLP polypeptide.

In another approach, soluble forms of hC/BTLP polypeptides still capable of binding the ligand in competition with endogenous hC/BTLP polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the hC/BTLP polypeptide.

In still another approach, expression of the gene encoding endogenous hC/BTLP polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of hC/BTLP and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates hC/BTLP polypeptide, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of hC/BTLP by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of hC/BTLP polypeptides in combination with a suitable pharmaceutical carrier.

Formulation and Administration

Peptides, such as the soluble form of hC/BTLP polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a

polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

5 All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

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SEQUENCE LISTING

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(1) GENERAL INFORMATION

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(i) APPLICANT: SmithKline Beecham Corporation

(ii) TITLE OF THE INVENTION: HUMAN CARDIAC/BRAIN TOLLOID-LIKE
PROTEIN

15

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:

20

(A) ADDRESSEE: SmithKline Beecham,
Corporate Intellectual Property

(B) STREET: Two New Horizons Court

(C) CITY: Brentford

25

(D) COUNTY: Middlesex

(E) COUNTRY: United Kingdom

(F) POST CODE: TW8 9EP

30

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: DOS

35

(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: TO BE ASSIGNED

40

(B) FILING DATE: 16-DEC-1997

(C) CLASSIFICATION: UNKNOWN

(vii) PRIOR APPLICATION DATA:

45

(A) APPLICATION NUMBER: 60/034,471

(B) FILING DATE: 02-JAN-1997

50

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: THOMPSON, Clive Beresford

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(C) REFERENCE/DOCKET NUMBER: ATG-50038

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(ix) TELECOMMUNICATION INFORMATION:

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(B) TELEFAX: +44 181 975 6294

(C) TELEX:

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(2) INFORMATION FOR SEQ ID NO:1:

15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5145 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

25 CTTACCTGCC CTCCGCCAC CCGTGGGCCC CTAGCCAACT TCTCCCTGCG ACTGGGGGTA 60
ACAGGCAGTG CTGCCCCTCT CTA CTGTCCC GGCGGCATCC ACATGTTTCC GGACACCTGA 120
GCACCCCGGT CCCGCCGAGG AGCCTCCGGG TGGGGAGAAG AGCACCAGTG CCCCTAGCCC 180
CGCACATCAG CGCGGACCGC GGCTGCCTAA CTTCTGGGTC CCGTCCCTTC CTTTCTCTCC 240
30 GGGGGAGGAG GATGGGGTTG GGAACGCTTT CCCCAGGAT GCTCGTGTGG CTGGTGGCCT 300
CGGGGATTGT TTTCTACGGG GAGCTATGGG TCTGCGCTGG CCTCGATTAT GATTACACTT 360
TTGATGGGAA CGAAGAGGAT AAAACAGAGA CTATAGATTA CAAGGACCCG TGTAAAGCCG 420
CTGTATTTTG GGGCGATATT GCCTTAGATG ATGAAGACTT AAATATCTTT CAAATAGATA 480
35 GGACAATTGA CCTTACGCAG AACCCCTTGG GAAACCTTGG ACATACCACA GGTGGACTTG 540
GAGACCATGC TATGTCAAAG AAGCGAGGGG CCTCTACCA ACTTATAGAC AGGATAAGAA 600
GAATTGGCTT TGGCTTGGAG CAAAACAACA CAGTTAAGGG AAAAGTACCT CTACAATTCT 660
CAGGGCAAAA TGAGAAAAAT CGAGTTCCCA GAGCCGCTAC ATCAAGAACG GAAAGAGTAT 720
GGCCTGGAGG CGTTATTCCT TATGTTATAG GAGGAACTT CACTGGCAGC CAGAGAGCCA 780
40 TGTTCAGCA GGCCATGAGG CACTGGGAAA AGCACACATG TGTGACTTTC ATAGAAAGAA 840
GTGATGAAGA GAGTTACATT GTATTCACCT ATAGGCCTTG TGGATGCTGC TCCTATGTAG 900
GTCGCGGAGG AAGTGGACCT CAGGCAATCT CTATCGGCAA GAACTGTGAT AAATTGGGA 960
TTGTTGTTCA TGAATTGGGT CATGTGATAG GCTTTTGGCA TGAACACACA AGACCAGATC 1020
45 GAGATAACCA CGTAACTATC ATAAGAGAAA ACATCCAGCC AGGTCAGAG TACAATTTTC 1080
TGAAGATGGA GCCTGGAGAA GCAAATCAC TTGGAGAAAG ATATGATTTT GACAGTATCA 1140
TGCACTATGC CAGGAACACC TTCTCAAGGG GGATGTTTCT GGATACCATC CTCCCTCCC 1200
GTGATGATAA TGGCATACGT CCTGCAATTG GTCAGCGAAC CCGTCTAAGC AAAGGAGATA 1260
50 TCGCACAGGC AAGAAAGCTG TATAGATGTC CAGCATGTGG AGAACTCTA CAAGAATCCA 1320
ATGGCAACCT TTCCTCTCCA GGATTCCCA ATGGCTACCC TTCCTACACA CACTGCATCT 1380
GGAGAGTTTC TGTGACCCCA GGGGAGAAGA TTGTTTAAA TTTTACAACG ATGGATCTAT 1440

55

	ACAAGAGTAG TTTGTGCTGG TATGACTATA TTGAAGTAAG AGACGGGTAC TGGAGAAAAT	1500
	CACCTCTCCT TGGTAGATTC TGTGGGGACA AATTGCCTGA AGTTCCTACT TCTACAGACA	1560
5	GCAGAATGTG GATTGAGTTT CGTAGCAGCA GTAATTGGGT AGGAAAAGGC TTTGCAGCTG	1620
	TCTATGAAGC GATCTGTGGA GGTGAGATAC GTAAAAATGA AGGACAGATT CAGTCTCCCA	1680
	ATTATCCTGA TGACTATCGC CCGATGAAGG AATGTGTGTG GAAAATAACA GTGTCTGAGA	1740
	GCTACCACGT CGGGCTGACC TTTCAGTCCT TTGAGATTGA AAGACATGAC AATTGTGCTT	1800
10	ATGACTACCT GGAAGTTAGA GATGGAACCA GTGAAAATAG CCCTTTGATA GGGCGTTTCT	1860
	GTGGTTATGA CAAACCTGAA GACATAAGAT CTACCTCCAA TACTTTGTGG ATGAAGTTTG	1920
	TTTCTGACGG AACTGTGAAC AAAGCAGGGT TTGCTGCTAA CTTTTTTAAA GAGGAAGATG	1980
	AGTGTGCCAA ACCTGACCCT GGAGGCTGTG AGCAGCGATG TCTGAACACT CTGGGCAGTT	2040
15	ACCACTGTGC CTGTGAGCCT GGCTATGAGC TGGGCCCAAG CAGAAGGAGC TGTGAAGCTG	2100
	CTTGTGGTGG ACTTCTTACC AAACCTAACG GCACCATAAC CACCCCTGGC TGGCCCAAGG	2160
	AGTACCCTCC TAATAAGAAC TGTGTGTGGC AAGTGGTTGC ACCAACCAG TACAGAAATT	2220
	CTGTGAAGTT TGAGTTTTTT GAATTGGAAG GCAATGAGGT TTGCAATAT GATTATGTGG	2280
20	AGATCTGGAG TGGTCTTTCC TCTGAGTCTA AACTGCATGG CAAATTCTGT GCGCTGAAG	2340
	TGCCTGAAGT GATCACATCC CAGTTCAACA ATATGAGAAT TGAATTCAAA TCTGACAATA	2400
	CTGTATCCAA GAAGGGCTTC AAAGCACATT TTTTCTCAGA CAAAGATGAA TGCTCTAAGG	2460
	ATAATGGTGG ATGTCAGCAC GAATGTGTCA ACACGATGGG GAGCTACATG TGTCAATGCC	2520
25	GTAATGGATT TGTGCTACAT GACAATAAAC ATGATTGCAA GGAAGCTGAG TGTGAACAGA	2580
	AGATCCACAG TCCAAGTGGC CTCATCACCA GTCCCAACTG GCCAGACAAG TACCCAAAGCA	2640
	GGAAAGAATG CACTTGGGAA ATCAGCGCCA CTCCTGGCCA CCGAATCAAA TTAGCCTTTA	2700
	GTGAATTGA GATTGAGCAG CATCAAGAAT GTGCTTATGA CCACCTAGAA GTATTTGATG	2760
30	GAGAAACAGA AAAGTCACCG ATTCTTGGAC GACTATGTGG CAACAAGATA CCAGATCCCC	2820
	TTGTGGCTAC TGGAAATAAA ATGTTTGTTC GGTTTGTTC TGATGCATCT GTTCAAAGAA	2880
	AAGGCTTTCA AGCCACACAT TCTACAGAGT GTGGCGGACG ATTGAAAGCA GAATCAAAAC	2940
	CAAGAGATCT GTACTCACAT GCTCAGTTTG GTGATAACAA CTACCCAGGA CAGGTGACT	3000
	GTGAATGGCT ATTAGTATCA GAACGGGGCT CTCGACTTGA ATTATCCTTC CAGACATTTG	3060
35	AAGTGGAGGA AGAAGCGGAC TGTGGCTATG ACTATGTGGA GCTCTTTGAT GGTCTTGATT	3120
	CAACAGCTGT GGGGCTTGGT CGATTCTGTG GATCCGGGCC ACCAGAAGAG ATTTATTCAA	3180
	TTGGAGATTC AGTTTTAATT CATTTCCACA CTGATGACAC AATCAACAAG AAGGGATTTT	3240
	ATATAAGATA CAAAAGCATA AGATATCCAG ATACCACACA TACCAAAAAA TAACACCAAA	3300
40	ACCTCTGTCA GAACACAAAG GAATGTGCAT AATGGAGAGA AGACATATTT TTTTAAAC	3360
	TGAAGATATT GGCACAAATG TTTTATACAA AGAGTTTGAA CAAAAAATCC CTGTAAGACC	3420
	AGAATTATCT TTGTACTAAA AGAGAAGTTT CCAGCAAAAC CCTCATCAGC ATTACAAGGA	3480
	TATTTGAACT CCATGCTTGA TGGTATTAAT AAAGCTGGTG AAAGGGCATC ATATACTTCA	3540
45	AGGAAGACTC TACAAGCTTT TGTTCACAGC TTGAAATAGA TGCCTCACAA TTCAGACAGT	3600
	TTAATTCAGG AACTGTGACC CTGAAGTGTT CTTTTGACA ATTTGTCAAG ATTTAGGGAC	3660
	ATAAAATGAT CTTGCAGGTC GTAAACTGGA AAACAGTATT TTGGTTGTCT TAGGATAATT	3720
	GCTGACTTTG TATCTTGGAT ACAGTGTAAG CCAGATCCAT ATAAGGTGAA TGTGAAATGG	3780
	GAGTCTTCTG AGGGTGATTT GTACTTTCCA TGTGTATGTG TGTGTCTGGT GTTTGGAAAC	3840
50	TGGGATATTT CAGCTTCATT ATTTCACATT GCAGGCCAGC TTAACCTCTG AAACACAAAT	3900
	GATCTTGAGA CCACTTTAGT GTACTTACAT TTAGATGAGT TTGAAATCTC AATGGTGTCT	3960
	AATTATTGCA GTTAAATTCT AGACATCAGT TCTTTAAGTC TCAGAAAACG CCCAGTGAAT	4020

	5	<p>TGGTAAACTT AGTTCCTTTT TTTGGAAGTG CTGCCCTTTC ACACCAAATC CAAGAAGCCT</p> <p>GTGATGTCTT ATGAACCTTA TGAGAAAACCT CCGAAGAGGT GTGAGCAGGA TTCTTCTGAA</p> <p>TGACTGTCTG GATGGTTCAT TACTCAAGTT ACTGCTGCTG CTATTGTCTT TCCTTTGTG</p> <p>TCGATCTGTT ATTGTTGTAT TATTATTGTT GATGTTGTCA TGGTTAATCT ATTTTTTAA</p> <p>ATTGAAATGA AGCAGAAGTA GGCCTTGTA GAACTGAAAG GTCTCTTTCA TTTTCTCTT</p> <p>CCTGGGATTC ATTTTTTCAA AACACAATGC TGGAAAAAAA AGATTGTGTT CTGAAAGACT</p> <p>TCTTATGGTG CTATTCCATA AACTTTTTTT CAAACAAGTT TTTGACCTTT GAGCCAACCC</p> <p>ACCCGTAGAC TACGAATGTC TCCTATGGC TGGTAGCATT TGAAGACTAA AGACTTGTC</p> <p>AATATATCAA GAGTATATCA TTGCAAGGGC AGCACTTGTC CTGTGGAACA ACTACTTATA</p> <p>ATGCCCTTAGA ATTCTGTCAC ATGATCAAAC AGATCCTCCT AAAACACACC TTTTGAAATG</p> <p>TTGAACATAA TAGTGATGTT TAATTAACAG CTCTATGAAG AAAATCCATT TCCATGACTG</p> <p>AAGCATTGGA TATAAATATG GTGTCCTGCT TTTTGTGTAG AAAATGTAAT TTGAGGATGA</p> <p>ATTTTCTGCT TTAAGGCAT GTGTGTTTTT AAAATTAATG AATGTAGATG TGTGATTGTC</p> <p>TGAGTGAGTG AAACATAAG AGGTAAAAAA TAATGGGTGG TTGAAAAGTT AAAATGTATG</p> <p>TGCCAAGTTC TACTAGAATT CCATTTGAAA TAGCACCTTC CTTAGGTTTC ATGGACAAAT</p> <p>AATGGGAAC TCTAATTTTG ATCAATCCCA TTAACAAAAA GCTCTTTCCT TTAGAGAAAC</p> <p>TCTATTTTGA TGTCAATATA GATTACTGTA TGAAGTAGCT TTGTGCTGCT TACCTGTCCA</p> <p>TGAGCATACA ACATTGAATA CAATTGGGTG TATTCTTTCA GTTTTACACA ATTAAGTAT</p> <p>ACACACAGAT GTAAAAAAA AAAAAAAAAC TCGAG</p>	<p>4080</p> <p>4140</p> <p>4200</p> <p>4260</p> <p>4320</p> <p>4380</p> <p>4440</p> <p>4500</p> <p>4560</p> <p>4620</p> <p>4680</p> <p>4740</p> <p>4800</p> <p>4860</p> <p>4920</p> <p>4980</p> <p>5040</p> <p>5100</p>
	25		5145

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1013 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

40	Met Gly Leu Gly Thr Leu Ser Pro Arg Met Leu Val Trp Leu Val Ala	
	1 5 10 15	
	Ser Gly Ile Val Phe Tyr Gly Glu Leu Trp Val Cys Ala Gly Leu Asp	
	20 25 30	
45	Tyr Asp Tyr Thr Phe Asp Gly Asn Glu Glu Asp Lys Thr Glu Thr Ile	
	35 40 45	
	Asp Tyr Lys Asp Pro Cys Lys Ala Ala Val Phe Trp Gly Asp Ile Ala	
	50 55 60	
50	Leu Asp Asp Glu Asp Leu Asn Ile Phe Gln Ile Asp Arg Thr Ile Asp	
	65 70 75 80	
	Leu Thr Gln Asn Pro Phe Gly Asn Leu Gly His Thr Thr Gly Gly Leu	
	85 90 95	

Gly Asp His Ala Met Ser Lys Lys Arg Gly Ala Leu Tyr Gln Leu Ile
 100 105 110
 5 Asp Arg Ile Arg Arg Ile Gly Phe Gly Leu Glu Gln Asn Asn Thr Val
 115 120 125
 Lys Gly Lys Val Pro Leu Gln Phe Ser Gly Gln Asn Glu Lys Asn Arg
 130 135 140
 10 Val Pro Arg Ala Ala Thr Ser Arg Thr Glu Arg Val Trp Pro Gly Gly
 145 150 155 160
 Val Ile Pro Tyr Val Ile Gly Gly Asn Phe Thr Gly Ser Gln Arg Ala
 165 170 175
 15 Met Phe Lys Gln Ala Met Arg His Trp Glu Lys His Thr Cys Val Thr
 180 185 190
 Phe Ile Glu Arg Ser Asp Glu Glu Ser Tyr Ile Val Phe Thr Tyr Arg
 195 200 205
 20 Pro Cys Gly Cys Cys Ser Tyr Val Gly Arg Arg Gly Ser Gly Pro Gln
 210 215 220
 Ala Ile Ser Ile Gly Lys Asn Cys Asp Lys Phe Gly Ile Val Val His
 225 230 235 240
 25 Glu Leu Gly His Val Ile Gly Phe Trp His Glu His Thr Arg Pro Asp
 245 250 255
 Arg Asp Asn His Val Thr Ile Ile Arg Glu Asn Ile Gln Pro Gly Gln
 260 265 270
 30 Glu Tyr Asn Phe Leu Lys Met Glu Pro Gly Glu Ala Asn Ser Leu Gly
 275 280 285
 Glu Arg Tyr Asp Phe Asp Ser Ile Met His Tyr Ala Arg Asn Thr Phe
 290 295 300
 Ser Arg Gly Met Phe Leu Asp Thr Ile Leu Pro Ser Arg Asp Asp Asn
 305 310 315 320
 35 Gly Ile Arg Pro Ala Ile Gly Gln Arg Thr Arg Leu Ser Lys Gly Asp
 325 330 335
 Ile Ala Gln Ala Arg Lys Leu Tyr Arg Cys Pro Ala Cys Gly Glu Thr
 340 345 350
 40 Leu Gln Glu Ser Asn Gly Asn Leu Ser Ser Pro Gly Phe Pro Asn Gly
 355 360 365
 Tyr Pro Ser Tyr Thr His Cys Ile Trp Arg Val Ser Val Thr Pro Gly
 370 375 380
 45 Glu Lys Ile Val Leu Asn Phe Thr Thr Met Asp Leu Tyr Lys Ser Ser
 385 390 395 400
 Leu Cys Trp Tyr Asp Tyr Ile Glu Val Arg Asp Gly Tyr Trp Arg Lys
 405 410 415
 50 Ser Pro Leu Leu Gly Arg Phe Cys Gly Asp Lys Leu Pro Glu Val Leu
 420 425 430
 Thr Ser Thr Asp Ser Arg Met Trp Ile Glu Phe Arg Ser Ser Ser Asn

435 440 445
 Trp Val Gly Lys Gly Phe Ala Val Tyr Glu Ala Ile Cys Gly Gly
 5 450 455 460
 Glu Ile Arg Lys Asn Glu Gly Gln Ile Gln Ser Pro Asn Tyr Pro Asp
 465 470 475 480
 Asp Tyr Arg Pro Met Lys Glu Cys Val Trp Lys Ile Thr Val Ser Glu
 10 485 490 495
 Ser Tyr His Val Gly Leu Thr Phe Gln Ser Phe Glu Ile Glu Arg His
 500 505 510
 Asp Asn Cys Ala Tyr Asp Tyr Leu Glu Val Arg Asp Gly Thr Ser Glu
 15 515 520 525
 Asn Ser Pro Leu Ile Gly Arg Phe Cys Gly Tyr Asp Lys Pro Glu Asp
 530 535 540
 Ile Arg Ser Thr Ser Asn Thr Leu Trp Met Lys Phe Val Ser Asp Gly
 20 545 550 555 560
 Thr Val Asn Lys Ala Gly Phe Ala Ala Asn Phe Phe Lys Glu Glu Asp
 565 570 575
 Glu Cys Ala Lys Pro Asp Arg Gly Gly Cys Glu Gln Arg Cys Leu Asn
 25 580 585 590
 Thr Leu Gly Ser Tyr Gln Cys Ala Cys Glu Pro Gly Tyr Glu Leu Gly
 595 600 605
 Pro Asp Arg Arg Ser Cys Glu Ala Ala Cys Gly Gly Leu Leu Thr Lys
 610 615 620
 Leu Asn Gly Thr Ile Thr Thr Pro Gly Trp Pro Lys Glu Tyr Pro Pro
 30 625 630 635 640
 Asn Lys Asn Cys Val Trp Gln Val Val Ala Pro Thr Gln Tyr Arg Ile
 645 650 655
 Ser Val Lys Phe Glu Phe Phe Glu Leu Glu Gly Asn Glu Val Cys Lys
 35 660 665 670
 Tyr Asp Tyr Val Glu Ile Trp Ser Gly Leu Ser Ser Glu Ser Lys Leu
 675 680 685
 His Gly Lys Phe Cys Gly Ala Glu Val Pro Glu Val Ile Thr Ser Gln
 40 690 695 700
 Phe Asn Asn Met Arg Ile Glu Phe Lys Ser Asp Asn Thr Val Ser Lys
 705 710 715 720
 Lys Gly Phe Lys Ala His Phe Phe Ser Asp Lys Asp Glu Cys Ser Lys
 45 725 730 735
 Asp Asn Gly Gly Cys Gln His Glu Cys Val Asn Thr Met Gly Ser Tyr
 740 745 750
 Met Cys Gln Cys Arg Asn Gly Phe Val Leu His Asp Asn Lys His Asp
 50 755 760 765
 Cys Lys Glu Ala Glu Cys Glu Gln Lys Ile His Ser Pro Ser Gly Leu
 770 775 780

5 Ile Thr Ser Pro Asn Trp Pro Asp Lys Tyr Pro Ser Arg Lys Glu Cys
 785 790 795 800
 Thr Trp Glu Ile Ser Ala Thr Pro Gly His Arg Ile Lys Leu Ala Phe
 805 810 815
 Ser Glu Phe Glu Ile Glu Gln His Gln Glu Cys Ala Tyr Asp His Leu
 820 825 830
 10 Glu Val Phe Asp Gly Glu Thr Glu Lys Ser Pro Ile Leu Gly Arg Leu
 835 840 845
 Cys Gly Asn Lys Ile Pro Asp Pro Leu Val Ala Thr Gly Asn Lys Met
 850 855 860
 15 Phe Val Arg Phe Val Ser Asp Ala Ser Val Gln Arg Lys Gly Phe Gln
 865 870 875 880
 Ala Thr His Ser Thr Glu Cys Gly Gly Arg Leu Lys Ala Glu Ser Lys
 885 890 895
 20 Pro Arg Asp Leu Tyr Ser His Ala Gln Phe Gly Asp Asn Asn Tyr Pro
 900 905 910
 Gly Gln Val Asp Cys Glu Trp Leu Leu Val Ser Glu Arg Gly Ser Arg
 915 920 925
 25 Leu Glu Leu Ser Phe Gln Thr Phe Glu Val Glu Glu Ala Asp Cys
 930 935 940
 Gly Tyr Asp Tyr Val Glu Leu Phe Asp Gly Leu Asp Ser Thr Ala Val
 945 950 955 960
 30 Gly Leu Gly Arg Phe Cys Gly Ser Gly Pro Pro Glu Glu Ile Tyr Ser
 965 970 975
 Ile Gly Asp Ser Val Leu Ile His Phe His Thr Asp Asp Thr Ile Asn
 980 985 990
 35 Lys Lys Gly Phe His Ile Arg Tyr Lys Ser Ile Arg Tyr Pro Asp Thr
 995 1000 1005
 Thr His Thr Lys Lys
 1010

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3690 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCGGCA CGAGCTCGTG CCGCTCGTGC CGCGGGTACT GGAGAAAATC ACCTCTCCTT

60

	GATTCTGTGG	GGACAAATTG	CCTGAAGTTC	TTACTTCTAC	AGACAGCAGA	ATGTGGATTG	120
	AGTTTCGTAG	CAGCAGTAAT	TGGGTAGGAA	AAGGCTTTGC	AGCTGTCTAT	GAAGCGATCT	180
5	GTGGAGGTGA	GATACGTAAA	AATGAAGGAC	AGATTCAGTC	TCCCAATTAT	CCTGATGACT	240
	ATCGCCCGAT	GAAAGAATGT	GTGTGGAAAA	TAACAGTGTC	TGAGAGCTAC	CACGTCGGGC	300
	TGACCTTTCA	GTCTTTTGAG	ATTGAAAGAC	ATGACAATTG	TGCTTATGAC	TACCTGGAAG	360
	TTAGAGATGG	AACCAGTGAA	AATAGCCCTT	TGATAGGGCG	TTTCTGTGGT	TATGACAAAC	420
10	CTGAAGACAT	AAGATCTACC	TCCAATACTT	TGTGGATGAA	GTTTGTCTT	GACGGAACTG	480
	TGAACAAAGC	AGGGTTTGCT	GCTAACTTTT	TTAAAGAGGA	AGATGAGTGT	GCCAAACCTG	540
	ACCGTGGAGG	CTGTGAGCAG	CGATGTCTGA	ACACTCTGGG	CAGTTACCAG	TGTGCCTGTG	600
	AGCCTGGCTA	TGAGCTGGGC	CCAGACAGAA	GGAGCTGTGA	AGCTGCTTGT	GGTGGACTTC	660
15	TTACCAAAT	TAACGGCACC	ATAACCACCC	CTGGCTGGCC	CAAGGAGTAC	CCTCCTAATA	720
	AGAACTGTGT	GTGGCAAGTG	GTTGCACCAA	CCCAGTACAG	AATTTCTGTG	AAGTTTGAGT	780
	TTTTTGAATT	GGAAGGCAAT	GAAGTTTGCA	AATATGATTA	TGTGGAGATC	TGGAGTGGTC	840
	TTTCTCTGA	GTCTAAACTG	CATGGCAAAT	TCTGTGGCGC	TGAAGTGCCT	GAAGTGATCA	900
20	CATCCCAGTT	CAACAATATG	AGAATTGAAT	TCAAATCTGA	CAATACTGTA	TCCAAGAAGG	960
	GCTTCAAAGC	ACATTTTTTC	TCAGACAAAG	ATGAATGCTC	TAAGGATAAT	GGTGGATGTC	1020
	AGCACGAATG	TGTCAACACG	ATGGGGAGCT	ACATGTGTCA	ATGCCGTAAT	GGATTTGTGC	1080
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25	GTGGCCTCAT	CACCAGTCCC	AACTGGCCAG	ACAAGTACCC	AAGCAGGAAA	GAATGCACTT	1200
	GGGAATCAG	CGCCACTCCT	GGCCACCGAA	TCAAATTAGC	CTTTAGTGAA	TTTGAGATTG	1260
	AGCAGCATCG	GGAATGTGCT	TATGACCACT	TAGAAGTATT	TGATGGAGAA	ACAGAAAAGT	1320
	CACCGATTCT	TGGACGACTA	TGTGGCAACA	AGATAACCAGA	TCCCCTTGTG	GCTACTGGAA	1380
30	ATAAAATGTT	TGTTTCGGTTT	GTTTCTGATG	CATCTGTTCA	AAGAAAAGGC	TTTCAAGCCA	1440
	CACATTCTAC	AGAGTGTGGC	GGACGATTGA	AAGCAGAATC	AAAACCAAGA	GATCTGTACT	1500
	CACATGCTCA	GTTTGGTGAT	AACAACCTACC	CAGGACAGGT	TGACTGTGAA	TGGCTATTAG	1560
	TATCAGAACG	GGGCTCTCGA	CTTGAATTAT	CCTTCCAGAC	ATTTGAAGTG	GAGGAAGAAG	1620
	CAGACTGTGG	CTATGACTAT	GTGGAGCTCT	TTGATGGTCT	TGATTCAACA	GCTGTGGGGC	1680
35	TTGGTCGATT	CTGTGGATCC	GGGCCACCCAG	AAGAGATTTA	TTCAATTGGA	GATTCACTTT	1740
	TAATTCATTT	CCCACTGAT	GACACAATCA	ACAAGAAGGG	ATTTTATATA	AGATACAAAA	1800
	GCATAAGATA	TCCAGATACC	ACACATACCA	AAAAATAACA	CCAAAACCTC	TGTCAGAACA	1860
	CAAAGGAATG	TGCATAATGG	AGAGAAGACA	TATTTTTTTT	AAAACCTGAAG	ATATTGGCAC	1920
40	AAATGTTTTA	TACAAAGAGT	TTGAACAAAA	AATCCCTGTA	AGACCAGAAT	TATCTTTGTA	1980
	CTAAAAGAGA	AGTTTCCAGC	AAAACCTCA	TCAGCATTAC	AAGGATATTT	GAACTCCATG	2040
	CTTGATGGTA	TTAATAAAGC	TGGTGAAAGG	GCATCATATA	CTTCAAGGAA	GACTCTACAA	2100
	GCTTTTGTTT	ACAGCTTGAA	ATAGATGCCT	CACAATTCAG	ACAGTTTAAAT	TCAGGAACTG	2160
45	TGACCCTGAA	GTGTTCTTTT	TGACAATTG	TCAAGATTTA	GGGACATAAA	ATGATCTTGC	2220
	AGGTCGTAAA	CTGGAAAACA	GTATTTTGGT	TGTCTTAGGA	TAATTGCTGA	CTTTGTATCT	2280
	TGGATACAGT	GTAACCCAGA	TCCATATAAG	GTGAATGTGA	AATGGGAGTC	TTCTGAGGGT	2340
	GATTTGTACT	TTCCATGTGT	ATGTGTGTGT	CTGGTGTGTT	GAAACTGGGA	TATTTAGCT	2400
50	TCATTATTTT	CACCTGCAGG	CCAGCTTAAC	CTCTGAAACA	CAAATGATCT	TGAGACCACT	2460
	TTAGTGTACT	TACATTTAGA	TGAGTTTGAA	ATCTCAATGG	TGTCTAATTA	TTGCAGTTAA	2520
	ATTCTAGACA	TCAGTCTTTT	AAGTCTCAGA	AAACGCCCCAG	TGAATTGGTA	AACCTAGTTC	2580
	TTTTTTTTTG	AAGTGCTGCC	TTTTACACCC	AAATCCAAGA	AGCCTGTGAT	GTCTTATGAA	2640

55

CCTTATGAGA AAACCTCCGAA GAGGTGTGAG CAGGATTCTT CTGAATGACT GTCTGGATGG 2700
 TTCATTACTC AAGTTACTGC TGCTGCTATT GTCTTTCCTT TGTGTGCGAT CTGTTATTGT 2760
 TGTATTATTA TTGTTGATGT TGTCAATGGT AATCTATTTT TTAATAATTGA AATGAAGCAG 2820
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 ATGTCTCCCT ATGGCTGTA GCAITTTGAAG ACTAAAGACT TGTCAAATAT ATCAAGAGTA 3060
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 GGCATGTGTG TTTTAAAAAT TAATGAATGT AGATGTGTGA TTGTCTGAGT GAGTGAACT 3360
 ACAAGAGGTA AAAAATAATG GGTGGTTGAA AAGTTAAAAT GTATGTGCCA AGTCTACTA 3420
 GAATTCATT TGAAATAGCA CCTTCCTTAG GTTTCATGGA CAAATAATGG GAACTTCTAA 3480
 TTTTGATCAA TCCCATTAAG AAAAGGCTCT TTCCTTTAGA GAACTCTAT TTTGATGTCA 3540
 ATATAGATTA CTGTATGAAG TAGCTTTGTG TCTGTTACCT GTCCATGAGC ATACAACATT 3600
 GAATCAATT GGGTGTATTC TTTCAGTTTT ACACAATTAA AGTATACACA CAGATGTAAA 3660
 AAAAAAAAAA AAAAAAAAAA AAAACTCGAG 3690

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 591 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Phe Cys Gly Asp Lys Leu Pro Glu Val Leu Thr Ser Thr Asp Ser Arg
 1 5 10 15
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 20 25 30
 Ala Ala Val Tyr Glu Ala Ile Cys Gly Gly Glu Ile Arg Lys Asn Glu
 35 40 45
 Gly Gln Ile Gln Ser Pro Asn Tyr Pro Asp Asp Tyr Arg Pro Met Lys
 50 55 60
 Glu Cys Val Trp Lys Ile Thr Val Ser Glu Ser Tyr His Val Gly Leu
 65 70 75 80
 Thr Phe Gln Ser Phe Glu Ile Glu Arg His Asp Asn Cys Ala Tyr Asp
 85 90 95
 Tyr Leu Glu Val Arg Asp Gly Thr Ser Glu Asn Ser Pro Leu Ile Gly

100 105 110
 Arg Phe Cys Gly Tyr Asp Lys Pro Glu Asp Ile Arg Ser Thr Ser Asn
 115 120 125
 Thr Leu Trp Met Lys Phe Val Ser Asp Gly Thr Val Asn Lys Ala Gly
 130 135 140
 Phe Ala Ala Asn Phe Phe Lys Glu Glu Asp Glu Cys Ala Lys Pro Asp
 145 150 155 160
 Arg Gly Gly Cys Glu Gln Arg Cys Leu Asn Thr Leu Gly Ser Tyr Gln
 165 170 175
 Cys Ala Cys Glu Pro Gly Tyr Glu Leu Gly Pro Asp Arg Arg Ser Cys
 180 185 190
 Glu Ala Ala Cys Gly Gly Leu Leu Thr Lys Leu Asn Gly Thr Ile Thr
 195 200 205
 Thr Pro Gly Trp Pro Lys Glu Tyr Pro Pro Asn Lys Asn Cys Val Trp
 210 215 220
 Gln Val Val Ala Pro Thr Gln Tyr Arg Ile Ser Val Lys Phe Glu Phe
 225 230 235 240
 Phe Glu Leu Glu Gly Asn Glu Val Cys Lys Tyr Asp Tyr Val Glu Ile
 245 250 255
 Trp Ser Gly Leu Ser Ser Glu Ser Lys Leu His Gly Lys Phe Cys Gly
 260 265 270
 Ala Glu Val Pro Glu Val Ile Thr Ser Gln Phe Asn Asn Met Arg Ile
 275 280 285
 Glu Phe Lys Ser Asp Asn Thr Val Ser Lys Lys Gly Phe Lys Ala His
 290 295 300
 Phe Phe Ser Asp Lys Asp Glu Cys Ser Lys Asp Asn Gly Gly Cys Gln
 305 310 315 320
 His Glu Cys Val Asn Thr Met Gly Ser Tyr Met Cys Gln Cys Arg Asn
 325 330 335
 Gly Phe Val Leu His Asp Asn Lys His Asp Cys Lys Glu Ala Glu Cys
 340 345 350
 Glu Gln Lys Ile His Ser Pro Ser Gly Leu Ile Thr Ser Pro Asn Trp
 355 360 365
 Pro Asp Lys Tyr Pro Ser Arg Lys Glu Cys Thr Trp Glu Ile Ser Ala
 370 375 380
 Thr Pro Gly His Arg Ile Lys Leu Ala Phe Ser Glu Phe Glu Ile Glu
 385 390 395 400
 Gln His Arg Glu Cys Ala Tyr Asp His Leu Glu Val Phe Asp Gly Glu
 405 410 415
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 420 425 430
 Asp Pro Leu Val Ala Thr Gly Asn Lys Met Phe Val Arg Phe Val Ser
 435 440 445

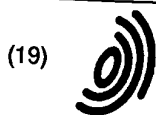
Asp Ala Ser Val Gln Arg Lys Gly Phe Gln Ala Thr His Ser Thr Glu
 450 455 460
 5 Cys Gly Gly Arg Leu Lys Ala Glu Ser Lys Pro Arg Asp Leu Tyr Ser
 465 470 475 480
 His Ala Gln Phe Gly Asp Asn Asn Tyr Pro Gly Gln Val Asp Cys Glu
 10 485 490 495
 Trp Leu Leu Val Ser Glu Arg Gly Ser Arg Leu Glu Leu Ser Phe Gln
 500 505 510
 Thr Phe Glu Val Glu Glu Glu Ala Asp Cys Gly Tyr Asp Tyr Val Glu
 15 515 520 525
 Leu Phe Asp Gly Leu Asp Ser Thr Ala Val Gly Leu Gly Arg Phe Cys
 530 535 540
 Gly Ser Gly Pro Pro Glu Glu Ile Tyr Ser Ile Gly Asp Ser Val Leu
 20 545 550 555 560
 Ile His Phe His Thr Asp Asp Thr Ile Asn Lys Lys Gly Phe His Ile
 565 570 575
 25 Arg Tyr Lys Ser Ile Arg Tyr Pro Asp Thr Thr His Thr Lys Lys
 580 585 590

30

Claims

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the hC/BTLP polypeptide of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.
2. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence contained in SEQ ID NO:1 encoding the hC/BTLP polypeptide of SEQ ID NO:2.
3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that is at least 80% identical to that of SEQ ID NO: 1 over its entire length.
4. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.
5. The polynucleotide of claim 1 which is DNA or RNA.
6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a hC/BTLP polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.
7. A host cell comprising the expression system of claim 6.
8. A process for producing a hC/BTLP polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.
9. A process for producing a cell which produces a hC/BTLP polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a hC/BTLP polypeptide.

10. A hC/BTLP polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.
11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.
12. An antibody immunospecific for the hC/BTLP polypeptide of claim 10.
13. A method for the treatment of a subject in need of enhanced activity or expression of hC/BTLP polypeptide of claim 10 comprising:
 - (a) administering to the subject a therapeutically effective amount of an agonist to said polypeptide; and/or
 - (b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the hC/BTLP polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said polypeptide activity *in vivo*.
14. A method for the treatment of a subject having need to inhibit activity or expression of hC/BTLP polypeptide of claim 10 comprising:
 - (a) administering to the subject a therapeutically effective amount of an antagonist to said polypeptide; and/or
 - (b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said polypeptide; and/or
 - (c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said polypeptide for its ligand, substrate, or receptor.
15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of hC/BTLP polypeptide of claim 10 in a subject comprising:
 - (a) determining the presence or absence of a mutation in the nucleotide sequence encoding said hC/BTLP polypeptide in the genome of said subject; and/or
 - (b) analyzing for the presence or amount of the hC/BTLP polypeptide expression in a sample derived from said subject.
16. A method for identifying compounds which inhibit (antagonize) or agonize the hC/BTLP polypeptide of claim 10 which comprises:
 - (a) contacting a candidate compound with cells which express the hC/BTLP polypeptide (or cell membrane expressing hC/BTLP polypeptide) or respond to hC/BTLP polypeptide; and
 - (b) observing the binding, or stimulation or inhibition of a functional response; or comparing the ability of the cells (or cell membrane) which were contacted with the candidate compounds with the same cells which were not contacted for hC/BTLP polypeptide activity.
17. An agonist identified by the method of claim 16.
18. An antagonist identified by the method of claim 16.
19. A recombinant host cell produced by a method of Claim 9 or a membrane thereof expressing a hC/BTLP polypeptide.



(19)

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European Patent Office

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(11)

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(12)

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(54) **Human cardiac/brain tolloid-like protein**

(57) HC/BTLP polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing hC/BTLP polypeptides and polynucleotides in the design of protocols for the treatment of restenosis, atherosclerosis, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), benign prostatic hypertrophy (BPH), nephritis, fibrosis, glomerulonephritis, gliosis, cirrhosis and anomalies of wound healing, such as keloids among others, and diagnostic assays for such conditions.

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European Patent
Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

EP 97 31 0521

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
P, X	WO 97 45528 A (WISCONSIN ALUMNI RES FOUND) 4 December 1997 (1997-12-04) * claims 5-8 *	1, 3, 5-7, 10, 11, 13	C12N15/57 C12N9/64 C07K14/51 A61K38/43
X	TAKAHARA K. ET AL.: "Bone Morphogenetic Protein-1 and a Mammalian Tolloid Homologue (mTld) Are Encoded by Alternatively Spliced Transcripts Which Are Differentially Expressed in Some Tissues" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 51, 23 December 1994 (1994-12-23), pages 32572-32578, XP002111748 * page 32574, column 2, line 10 - line 48; figures 3, 4 *	1-12, 19	
A	BOND J. S. ET AL.: "The astacin family of metalloendopeptidases" PROTEIN SCIENCE, vol. 4, no. 7, - July 1995 (1995-07) pages 1247-1261, XP002111749 * the whole document *	1-6, 10, 11	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
			C12N C07K A61K
INCOMPLETE SEARCH			
The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.			
Claims searched completely :			
Claims searched incompletely :			
Claims not searched :			
Reason for the limitation of the search: see sheet C			
Place of search BERLIN		Date of completion of the search 11 August 1999	Examiner Schönwasser, D
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

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ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.

EP 97 31 0521

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
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11-08-1999

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EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82